Graphite Oxide Nanoparticles Larger than 20nm in Diameter are Biocompatible with Mouse Embryonic Stem Cells

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Statement of Purpose: Embryonic stem cells (ESCs) have demonstrated pluripotency and to regenerate functional tissues[1-2]. However, transplantation of the ESCs *in vivo* is hindered by suboptimal cell survival post transplantation, compromising their restorative potential[3]. The goal of this study is to increase post-transplantation ESC survival. Graphite oxide (GO) nanoparticles in their readily synthesized form are water soluble single layered sheets ranging in size from 3 ηm to 6 μm in diameter[4]. They allow electrical insulation, remain highly economical, and provide easy fabrication[4]. With proper functionalization, hydrophobic biomolecules are loaded onto the surface through *pi-pi* stacking, allowing delivery of biomolecules to increase ESC survival following cell transplantation[4]. The 2 objectives are: 1) examine the effects of GO particles on mouse ESC viability, proliferation, and gene expressions, and 2) identify the optimal size and concentration of GO particles on mESC.

Methods: Cell and Cell Culture: Mouse embryonic stem cell line E14 was derived from inbred mouse strain 129/Ola, and transduced with a reporter gene over-expressing luciferase. The cells were cultured on gelatinized surface in media previously conditioned by mouse embryonic fibroblasts (MEF), consisting of DMEM (Gibco) with 20% FBS (Hyclone), 1% NEAA and 1% P/S (Invitrogen). Graphite Oxide: Graphite Oxide (GO) was synthesized using a modified Hummer's Method[4]. In order to control the size of the GO, varying levels of sonication and centrifugation were used. Small GO was bath sonicated with branched Poly(ethylene) glycol for 1 hour and centrifuged at 22,000g for 6 hrs; Medium GO was sonicated for 20 min and centrifuged at 22,000g for 30 min; Large GO was sonicated for 1 min and centrifuged at 7200g for 5 min. Study Design: The mESCs were seeded at 25,000 cells/cm^2. GO particles of three size ranges (small: d=3-20 ηm; medium: d=20-75; large: d=125-700) were dissolved in media at 0.005 mg/ml (low) or 0.01 mg/ml (high). Control group received only the media and experimental groups received media containing GO of different size ranges and concentrations, resulting in a total of six groups: Small (size)/Low (concentration), Small/High, Medium/Low, Medium/High, Large/Low, Large/High. End Point Analyses: At day 1, 3, and 7, cell viability (Live-Dead stain), growth (Picogreen DNA quantification), reporter gene expression (bioluminescence imaging), gene expressions of pluripotency markers Sac4, Sox2, Nanog (RT-PCR), and GO particle uptake (fluorescence microscopy) were evaluated. Statistical Analysis: 2-way ANOVA and Tukey-Kramer post-hoc test (*p<0.05).

Results: Atomic Force Microscopy images of readily synthesized GO particles clearly showed three different size ranges (Fig. 1). After 24 hours of culture, uptake of GO particles was found in all groups (Fig. 2A). All cells grew over time. However, cells receiving small GO showed less proliferation and more cell death (Fig. 2B and 3). 0.01 mg/ml of GO decreased cell growth. Furthermore, small GO significantly decreased luciferase expression over time compared to all other sizes (Fig. 4). No difference was found in the expressions of the pluripotency genes (Fig. 5).

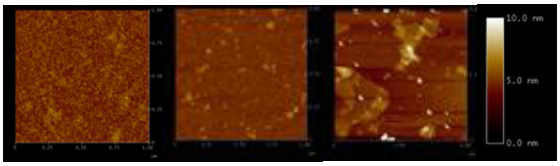

Fig.1. Atomic Force Microscopy Imaging of as-made GO particles.

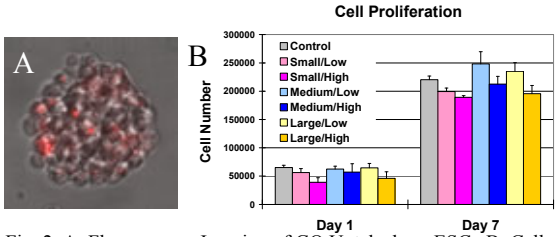

Fig. 2. A: Fluorescence Imaging of GO Uptake by mESC. B: Cell Proliferation over Time.

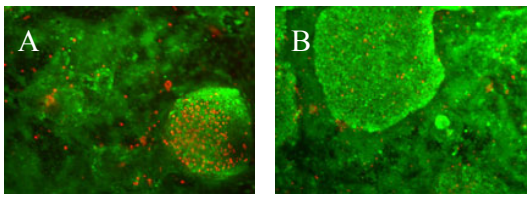

Fig. 3. A: Live-Dead Stain after 7 Days of Culture. 10x. A: small/high, B: large/high. Live cells stain green while dead cells stain red.

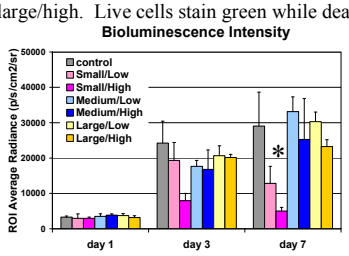

Fig. 4. Bioluminescence Intensity over Time.

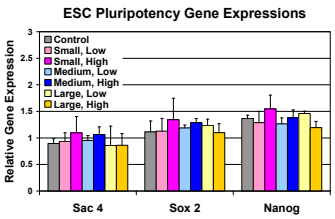

Fig. 5. Expressions of pluripotency genes Sac4, Sox2 and Nanog of mESC after day 7.

Conclusions: Small GO particles with diameter <20 nm suppressed cell growth, luciferase gene expression, and increased cell death. Higher concentrations of GO in all sizes also had detrimental effects on mESC growth. GO particles larger than 20 nm in 0.005 mg/ml are biocompatible and can be safely used for mESCs.

References: 1)Yang L, Nature. 2008 ;453(7194) :524-8. 2) Gong G, J Cell Physiol. 2010;224(3):664-71. 3) Smart N, Cir Res. 2008 ;23 :102(10) :1155-68. 4) Sun X, Nano Res. 2008 ; 1 :203-212.